TOTAL AMOUNT OF PROTEIN IN *LILIUM LONGIFLORUM* THUNB., EASTER LILY, STYLES FROM 2 DAYS PRE-ANTHESIS TO 10 DAYS POST-ANTHESIS

by

ABDULLAH SALIH AL-GHAMDI

B. S., University of Riyad, Riyad, 1975

A MASTER'S THESIS

submitted in partial fulfillment of the

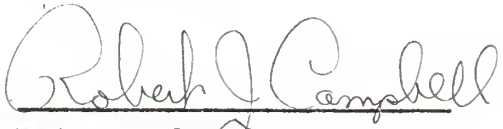
requirements for the degree

MASTER OF SCIENCE

Department of Horticulture

KANSAS STATE UNIVERSITY
Manhattan, Kansas
1979

Approved by:



Major Professor

Spec Coll
LD
2665
.T4
1979
A42
C.2

TABLE OF CONTENTS

Fig. 11. The total amount of protein in mg protein/style for non-heat-treated 'Nellie White' styles (control) and 'Nellie White' styles submerged for 5 min in 50°C water plotted against incubation time in hr. Both treated and non-treated styles were incubated at 24°C for 0, 12, 24 or 48 hr. Each data point represents the average of 3 separate replications and 3 determinations on each.

Fig. 12. The percentage nitrogen per 250 mg tissue determined by microkjeldahl for 'Ace' and 'Nellie White' styles plotted against the floral age in days. Each data point represents the average of 2 separate replications. The standard deviation ranged from $\pm$ 0.092% N to $\pm$ 0.007% N for 'Ace' and $\pm$ 0.078% N to $\pm$ 0.007% N for 'Nellie White'.

Fig. 13. The total amount of nitrogen in mg N/style determined by microkjeldahl on 'Ace' and 'Nellie White' styles plotted against the floral age in days. Each data point represents the average of 2 separate replications. The standard deviation ranged from $\pm$ 0.046 mg N/style to $\pm$ 0.003 mg N/style for 'Ace' and $\pm$ .033 mg N/style and $\pm$ 0.003 mg N/style for 'Nellie White'.

ACKNOWLEDGMENTS

I wish to extend my sincere thanks to Dr. Robert J. Campbell, my major professor, for his guidance, patience, technical expertise, and the considerable amount of time spent on going through my thesis.

My sincere thanks to the members of my committee, Dr. Carl D. Clayberg, Professor of Horticulture, Dr. Richard Mattson, Professor of Horticulture.

I gratefully acknowledge my employer, King Faisal University, Saudi Arabia, for the financial assistance.

To my parents, and my wife for their support and encouragement throughout my study and to others who have helped in this research yet are not mentioned, I also wish to express my appreciation.

INTRODUCTORY STATEMENT

This thesis has been written in manuscript form, for submission for publication in Theoretical and Applied Genetics. This research was conducted from the spring of 1978 through the spring 1979, in the Department of Horticulture laboratories at Kansas State University.

Total amount of protein in Lilium longiflorum Thunb., Easter lily, styles from 2 days pre-anthesis to 10 days post-anthesis.[1]

Abdullah S. Al-Ghamdi and Robert J. Campbell

Summary. Proteins were extracted using 2N NaOH from freeze dried powder obtained from Lilium longiflorum Thunb., Easter lily, styles from 2 days pre-anthesis until 10 days post-anthesis, and analyzed by using a colorimetric test; the powder was also analyzed for nitrogen by microkjeldahl.

Fresh and dry stylar weight increased in different ways as the flowers aged. Fresh weight increased most rapidly during the day preceding anthesis; increased less sharply after that, peaking at 9-10 days after anthesis; and then rapidly declined. Dry weight, about 9% of style fresh weight, was more variable than fresh weight, with a slight increase in dry weight the day of anthesis and after the fifth day after anthesis.

Total protein decreased. The largest amount of protein was in the two days pre-anthesis to day of anthesis styles. A sharp decrease in total amount of protein occurred

---

[1]Contribution No. _____ _____ _____-J, Department of Horticulture, Kansas Agricultural Experiment Station, Kansas State University, Manhattan, Kansas 66506.

from the day of anthesis to one day later. From one day after anthesis until the fifth day, the total protein seemed to plateau. From the fifth day until the ninth day the total protein decreased sharply.

Total protein in non heat-treated styles began at the same protein level expected for 1 day old styles, then showed the plateauing of protein across time as in 2 and 3 day old styles. From 24 hr up to 48 hr incubation there was a decrease in the amount of stylar protein.

The amount of protein in heat-treated styles was the same at 0 time and 12 hr as for non heat-treated styles but increased until it reached a high value at 24 hr incubation. By 48 hr, the amount decreased.

In the microkjeldahl assay for determining total nitrogen in styles, nitrogen amounts started at a relatively low level and increased slightly from two days pre-anthesis to 3 days post-anthesis. From the third day until the seventh day after anthesis, there was a sharp increase. % nitrogen then plateaued with increased variability.

Introduction:

Lilium longiflorum Thunb, the Easter lily, possesses a self-incompatibility reaction that prevents self seed set. Self incompatibility is controlled by a single gene locus. When the gene in the pollen tube matches one of the two in the style, the pollen tube will grow as an incompatible tube reaching half way down the 100 mm lily style in 48 hr with incubation at 24°C. When the pollen tube gene is unmatched by one in the style, the pollen tube will grow as a compatible reaching almost all the way down the style (D. de Nettancourt, 1977, Ascher, 1975).

Self incompatibility is due to a substance that moves from the style into the stylar canal during and after anthesis and blocks compatible pollen tube growth (Ascher, 1975).

The pattern of pollen tube growth in detached styles of Lilium longiflorum Thunb. varies with the physiological age of the style (Ascher and Peleoquin, 1966). In styles removed from buds, both compatible and incompatible pollen tubes grow at the same rate covering about 25 mm or $\frac{1}{4}$ of the distance covered by compatible tubes growing in styles from post anthesis flowers. At this stage there is neither an incompatibility reaction or long pollen tube growth since the style can not support either yet.

From anthesis and for the next 5 days, incompatible pollen tubes reach about one-half the length of compatible pollen tubes or 50 mm. During this interval in which pollination usually occurs, the self-incompatibility reaction appears strongest, the stigma begins to exude a substance especially suitable for pollen germination, the stylar canal cells secrete stylar exudate, the cells lining the canal form a callose coat, the style continues to elongate, and the stylar canal cells become endomitotic (Ascher, P. D., Personal Communication).

From 6 to 9 days after anthesis, there is no incompatibility reaction; the incompatible pollen tubes grow up to more than 3/4 the length of compatible pollen tubes, while in vivo pollination at this stage with incompatible pollen produces seed (Ascher 1966). During this interval the style stops actively secreting stylar canal substances, and by usually the ninth day has ceased exuding stigmatic exudate.

From 10 days after anthesis onward the length of both compatible and incompatible pollen tubes is the same, but the length they attain rapidly decreases. The style is senescing and no longer can support pollen tube growth.

These variations in the incompatibility of the incompatible pollen mean that the incompatibility reaction does or does not operate at various physiological ages of the styles.

The Easter lily has a hollow style lined with specialized epithelial cells on which pollen tubes grow to the ovary. The style provides the food and the environment for pollen tubes to grow. The style produces a particular substance called a special stylar substance for pollen tubes that can be used to advantage only by compatible pollen tubes; without it the growth of pollen tubes becomes incompatible (Ascher, 1975). The cells lining the stylar canal are densely cytoplasmic, rich in organelles and have an elaborately domed region (secretion zone) facing the canal that possesses ingrowths or paramural bodies. The substance these cells secrete moves from dictyosome cisternae in vesicles formed from the dictyosomes to the cell wall (Rosen and Thomas, 1970). Before anthesis there is a cuticle over the secretion zone of canal cell that may prevent release of secretion product.

Proteins and amino acids have been implicated in pollen tube growth and incompatibility. In Oenthera missouriensis the main difference between self- and cross-pollinated styles is the level and presence of Pro and Glu in the pool of free amino acids, suggesting that the main part of the free amino acids are used for metabolic purposes during pollen tube penetrance through the style. However, specific consumption of amino acids in connection with the synthesis of proteins which may be responsible for the incompatibility reaction are overshadowed

by the mass shift of the metabolic amino acid pool (Linder and Linskens, 1972).

In Nicotiana alata, the growth of pollen tubes evokes changes in the level of free amino acids in the style. The most marked changes are increases in the level of some amino acids; these increases are quantitively in direct ratio to the rate of pollen tube growth, which is much greater following compatible pollination. A reduction in the level of sugars accompanied by an accumulation of amino acid (asparagine) in pollinated styles was more rapid after compatible than incompatible pollination. This may have been a result of more intensive consumption of proteins during respiration when there was a scarcity of sugars in the style. The pollen tubes use substrates from the style tissue for respiration and that process is limited in the case of incompatible inhibition (Tupy, 1961).

Van der Donk (1975 a, b) found the RNA and proteins from virgin styles totally ineffective against pollen tube growth in vivo. But, the proteins of incompatible pollinated styles inhibited pollen tube growth but proteins from compatible pollinated styles stimulated pollen tube growth. Van der Donk obtained proteins active in pollen tube growth by injecting the RNA of pollinated styles into egg cells of Xenopus laevis. These proteins stimulated the growth of pollen tubes if S alleles of pollen tube and style were not identical, and reduced

pollen tube length where S-alleles were identical.

Protein synthesis is needed in lily styles, for the incompatibility reaction and for its support of pollen tube growth (Ascher, 1974).

Production of the stylar part of the self-incompatibility reaction in Easter lily is sensitive to heat and to inhibitors of protein synthesis and RNA synthesis. Stylar heat treatment given by dipping the styles in $50^{o}C$ water for 5-6 min followed by pollination causes all pollen tubes to grow as if compatible. Heat inactivates the incompatibility reaction, apparently by disrupting stylar metabolic process. The heat treatment also causes a release of the special stylar substance into the stylar canal (Hopper, Ascher and Peloquin, 1967).

Puromycin, a protein synthesis inhibitor (Ascher, 1974), injected at 1×10^{-3} M into Easter lily styles before mid-day on the day of anthesis, resulted in complete removal of the self-incompatible reaction. Both compatible and incompatible pollen tubes in treated styles reached the length of compatible tube growth in control styles. However treatment after mid-day had no effect on pollen tube growth.

Incompatible pollen tubes grew as compatible when styles were injected with a RNA synthesis inhibitor, 6-methyl purine at 1×10^{-3} M at anthesis, and one day post anthesis, and slightly less at the 2 day post anthesis.

6-methyl purine did not affect compatible pollen tube growth.

Susceptibility to puromycin treatment up to mid-day of anthesis suggests that a protein synthesis necessary for self incompatibility is not needed after anthesis, therefore the protein made is long-lived; susceptibility to 6-methyl purine treatment from anthesis to at least 2 days later would suggest that RNA needed for the incompatibility reaction is not long-lived. Puromycin treatment might be blocking the synthesis of RNA polymerase (Ascher, 1974).

The potent protein synthesis inhibitor cycloheximide suspended in stigmatic exudate and injected into lily styles post pollination caused both incompatible and compatible pollen tubes to cease growth just after entering the stylar canal; apparently protein synthesis is necessary for pollen tube growth (Ascher and Drewlow, 1970).

The purpose of this experiment was to determine the total amount of protein in two day pre-anthesis up to 10 days post-anthesis styles of Easter lily, Lilium longiflorum. We also wanted to determine the total amount of protein in lily styles given a heat treatment at one day post-anthesis to remove the self-incompatibility reaction and then incubated 0, 12, 24 or 48 hr.

Material and Method:

During May 1978, flowers of greenhouse-grown Lilium-longiflorum cultivars Ace and Nellie White (NW) were cut early the day of anthesis, or buds were cut 1-2 days pre-anthesis, and placed in jars of tap water on laboratory benches at room temperature under ceiling fluorescent light giving a light period of at least 14 hr a day. Stigma-styles were removed from the flowers and buds by cutting through the ovary with a triangular needle, and the remaining portion of the ovary snapped off.

We did not know which of the pre-anthesis buds would be one or two day pre-anthesis, so the stigma-style were cut out immediately from the buds without damage to them and both the buds and the styles labeled. The buds with styles removed were saved until they opened to determine which styles were one or two day pre-anthesis.

In the first experiment, we wanted to determine the total amount of protein in Easter lily styles across time from two days pre-anthesis up to 10 days post-anthesis. We collected 1687 'Ace' and 1587 'NW' styles distributed between 13 days. The number of styles was different from day to day, depending on how many flowers we had available in the greenhouse. We collected each age flower more than once.

In the second experiment we wanted to determine the affect of heat-treatment on the total amount of protein in styles treated at one day post-anthesis and incubated up to 2 days. 527 'Ace' and 256 'NW' styles were collected and distributed in almost equal amounts between 8 group/cultivar. Each group was weighed, and 4 groups/cultivar submerged in 50° C distilled water for 5 min. Treated and non-treated styles were incubated in 8 150 mm glass petri-dishes plates/cultivar on moist filter paper for 0, 12, 24 or 48 hr at 24°C.

All the various aged, heated and non-heated treated styles were weighed fresh, broken in two, frozen in liquid nitrogen, and then freeze-dried for either at least 12 hr in a Virtis Model 5135 B20 with dry ice and acetone in the chamber or at least 16 hr in a Virtis Automatic Freeze-Dryer Model No. 10-010, or until the samples became dry.

The freeze-dried styles were stored in a freezer at -20°C. Before grinding, the styles were weighed again for dry weight, and powdered in a Wiley Mill Model 3383-L10 at room temperature using a 60 mesh screen. The powder was stored in tightly sealed plastic containers inside a dessicator at -20° until chemical analysis.

Bradford (1976) recently published a simple, rapid, sensitive and inexpensive method for quantitation of protein with Coomassie Brilliant Blue G-250 (GB), based on the color shift that occurs when the dye binds to

protein. In that work, 5 mg protein/ml of test solution gave an absorbance of 0.275 units, which was five times as sensitive as the Lowry procedure. Slightly different absorbance values resulted when equal quantities of different protein reacted with CB (Bradford 1976). This method was adapted for use on stylar protein.

Reagent dye preparation:

100 mg of Coomassie Brilliant Blue G-250 (Sigma Chemical Co.) was dissolved in 50 ml 95% ethanol. To this solution, 100 ml 85% (w/v) phosphoric acid was added. The resulting solution was diluted to a final volume of 1 liter with distilled water. Final concentrations in the reagent were 0.01% (w/v) Coomassie Brilliant Blue G-250, 4.7% (w/v) ethanol and 8.5% (w/v) phosphoric acid. The solution was filtered using Whatman No. 41 and filtered again using a Millipore Filter Type GS 0.22 um until the solution was brown and clear and not blue.

Standard curve:

For standard curves to determine mg protein in an unknown, 100 mg bovine serum albumin, fraction V (BSA) (Sigma Chemical Co.) was dissolved in 0.15 M NaCl and made up to one liter. This protein solution in volumes up to 0.1 ml and containing 10 to 100 mg protein was pipetted into 12 x 100 mm test tubes; the volume was adjusted to 0.1 ml with 0.15M NaCl. Five ml of protein reagent was added to the test tube and the contents mixed by a

vortex mixer.

The binding of the dye to protein shifts the absorption maximum of the dye from 465 to 585 mm. The 585 mm peak reaches a maximum within 10 min of adding the protein reagent. Absorbance at 585 mm was measured after 10 min in a 3 ml optical glass (Fisher) cuvette in a Beckman Spectrophotometer Model 25 against a reagent blank prepared from 0.1 ml of the 0.15 M NaCl and 5 ml of protein reagent.

Mg protein was plotted against the corresponding absorbance. The standard curve was done 8 times, once each time protein was determined in the unknowns, and each point on the curve was done only once for each standard curve. The data was combined together into 1 curve since the 8 curves differed little. The average of the data was subjected to a simple linear regression analysis that resulted in a mathematical equation that allowed us to directly estimate the amount of protein from the absorbance value, and eliminated the necessity of reading protein values directly from a plotted curve.

Protein assay of stylar powder:

To determine the amount of protein in a stylar sample, we placed 15 mg of the dry powder in a 12 x 100 mm glass test tube. While mixing the dried powder on a Vortex mixer, we added 2 ml of 2N NaOH and let the test tube sit for 2 hr at room temperature. Five min before the time finished we filtered the solution on Whatman No. 1.

0.1 ml of the filtered solution was pipetted into 12 x 100 mm test tubes. Five ml of protein reagent was added to the test tube and the contents mixed by a vortex mixer. After 10 min absorbance at 585 mm was measured as before.

The protein assay of an unknown sample was done in 3 separate extraction replications, each one using 15 mg dry sample and 2 ml 2N NaOH. From each extraction we took 3 separate determinations, each one using 0.1 ml of the filtered solution and 5 ml of the protein reagent.

The amount of protein was calculated based either on 1 gr of dry tissue powder or on dry weight of tissue powder per style.

The total amount of nitrogen in the sample from 2 days pre-anthesis up to 10 days post-anthesis was determined using a microkjeldahl method and a Technicon Autoanalyzer II in the Soil Test laboratory in the Agronomy Department, Kansas State University, at Manhattan, Kansas. 250 mg of the powder was placed into 50 ml digestion tubes and 2 ml concentrated H_2SO_4 and 1 ml H_2O_2 added. The tubes were placed on a digestion block and heated at 375° C for 45 min, removed and allowed to cool about 15 min. 1 ml H_2O_2 was added and the tubes were reheated. If not clear, 1 ml more of H_2O_2 was added and the solution reheated until the solution became clear. The clear solution was brought up to 50 ml volume with distilled

water, and a 5 ml aliquot taken for N determination in the Technicon Autoanalyzer II. This was done on 2 250 mg sample replications.

This microkjeldahl assay is based on a colorimetric method in which an emerald-green color is formed by reaction of ammonia, sodium salicylate, sodium nitroprusside and sodium hypochlorite (chlorine source) in a buffered alkaline medium at pH of 12.8-13.00. The ammonia-salicylate complex is read at 666nm.

Result:

In the plot of protein concentration against absorbance (Fig. 1), the standard curve approached linearity over the range of 0 to 100 mg. The data was subjected to simple linear regression analysis that resulted in a mathematical equation that directly gave the amount of protein from the absorbance value and eliminated the necessity of reading protein concentration values directly from the standard curve. Departures from linearity on the curve are due to an overlap in spectrum of the two colors of the dye in solution (Bradford, 1976).

The fresh and dry weight of styles for both cultivars Ace and NW increased as the flowers got older, but in different ways (Fig. 2-3). The most rapid increase of fresh weight was during the day preceding anthesis, as styles went from 400 mg to 521 mg/style in 'Ace', and from 417 mg to 471 mg/style in 'NW'. After that, stylar fresh weight increased less sharply to a peak, either 9 days after anthesis in 'Ace' reaching 561 mg/style or at 8 days after anthesis in 'NW' reaching 545 mg/style. After that, stylar fresh weight for both cultivars rapidly declined, decreasing sharply from 561 mg to 508 mg in 'Ace', and from 545 mg to 480 mg/style in 'NW'.

Dry weight, about 9% of style weight, had more variation than fresh weight. There was a slight increase

in 'Ace' dry weight the day of anthesis and for both cultivars after the fifth day after anthesis, but in general the increase was small (Fig. 3). The ultimate number of cells in the style are laid down before the flower opens and do not increase in number as the style ages; at the same time, the length and the mass of the style increases. Consequently, the fresh weight should increase but the dry weight should remain level.

The total amount of protein in both 'Ace' and 'NW' styles on a per gram dry weight and on a per style basis decreased (Fig. 4, 5, 6 and 7). The largest amount of protein in styles of 'Ace' was from buds 2 days before anthesis and from flowers the day of anthesis, both at about 120 ug/g or 5.5 ug/style. There was a drop of protein in 'Ace' styles from 120 ug/g in 2 day pre-anthesis buds to 107 ug/g or from 5.5 ug/style to 4.5 ug/style in 1 day before-anthesis buds. The total amount of protein in styles of 'NW' was highest in the 2 days pre-anthesis to day of anthesis styles, with about 115 ug/g tissue or 4.8 ug/style. There was no large drop in 1 day pre-anthesis buds as there was in 'Ace'.

The total amount of protein sharply decreased from the day of anthesis to one day later both in 'Ace' styles, dropping from 119 ug/g tissue to 108 ug/g tissue or 5.6 ug/style to 4.5 ug/style, and in 'NW' styles, dropping from 115 to 105 ug/g tissue or 4.9 to 4.1 ug/style.

From one day after anthesis until the fifth day in 'Ace' styles, the total amount of protein seemed to plateau at about 106 ug/g tissue on a per gram basis or even to increase slightly to about 4.6 ug/style on a per style basis. From the fifth day until the ninth day the total amount of protein decreased sharply from 110 ug/g tissue to 83 ug/g tissue or from 4.6 ug/style to 4.0 ug/style (Fig. 4, 5).

From one day after anthesis until the fifth day in styles of 'NW', the total amount of protein plateaued at about 104 ug/g tissue or, if plotted as g/style, increased across time to the sixth day to 4.6 ug/style. The total amount of protein/g tissue after the fifth day until the tenth day decreased sharply from 104 ug to 82 ug/g tissue, but the ug protein/style decreased from the sixth day post-anthesis until the tenth day, from 4.6 ug to 3.7 ug/style (Fig. 6-7).

The amount of protein in the non heat-treated 'Ace' and 'NW' styles (control) began at the same protein level expected for 1 day old styles, and even though incubated in petri plates instead of being left in flowers showed the plateauing of protein across time as in 2 and 3 day old 'Ace' or 'NW' styles left in flowers. There was about 111 ug protein/g tissue or 4.8 ug/style in 'Ace' styles and 106 ug/g tissue or 4.5 ug/style in 'NW' styles.

The amount of protein in 'Ace' style continued at about the same level without much change until at 48 hr of incubation when the amount decreased slightly to about 103 ug/g tissue or 4.1 ug/style. In 'NW' there was a slight increase during the first 12 hr from 106 ug/g tissue to 110 ug/g tissue but, on a per style basis, there was not much change. From 24 hr up to 48 hr incubation, there was a decrease in the amount of stylar protein, from 110 ug/g tissue or 4.4 ug/style to 79 ug/g tissue or 3.8 ug/style.

The total amount of protein in heat-treated 'Ace' and 'NW' styles was the same at 0 time and 12 hr as for non heat-treated styles but then increased until it reached a high value at 24 hr incubation in 'Ace' styles of 127 ug/g tissue or 5.2 ug/style and in 'NW' styles of 111 ug/g or 4.6 ug/style. By 48 hr the amount decreased in 'Ace' styles to 116 ug/g tissue or 4.6 ug/style, and in 'NW' styles to 111 ug/g tissue or 4.4 ug/style (Fig. 8, 9).

In the microkjeldahl assay for determining the total amount of nitrogen in the styles (Fig. 12, 13), we found similar curves for both 'Ace' and 'NW' styles. Nitrogen amount in the 2 cultivars started at about the same level and increased slightly from two days pre-anthesis to 3 days post-anthesis, going from 2.02% N or 0.93 mg N/style up to 2.33% N or 1.03 mg N/style for 'Ace' and from 2.23% N or .93 mg N/style up to 2.59% N or 1.13

mg/style for 'NW'. From the third day until the seventh day after anthesis, there was a sharp increase from 2.33% N or 1.03 mg N/style to a high of 4.0% N or 1.84 mg N/style for 'Ace' and an increase from 2.59% N or 1.13 mg N/style up to 4.03% N or 1.86 mg/styles for 'NW'. % nitrogen then plateaued with increased variation at about 4% N or 1.84 mg N/style from 7 to 10 days after anthesis for both cultivars.

Discussion:

Some time in a plant's life cycle, proteins reach a constant state of turnover, a steady state condition where protein content remains constant because the rate of synthesis is equal to the rate of degradation. However, in the leaves of annual plants at least, the protein content reaches a maximum at the time the leaf becomes fully expanded and thereafter declines (Beevers, 1970). A reduced protein content of the styles could be the result of either a reduced synthetic capacity or an increased protein degradation depending on stylar age. Experimental evidence from detached leaves supports both these possibilities. But, senescence is attributable to an increased degradation (Kurashi, 1968).

Before anthesis, lily buds are undergoing rapid change with such events as microspore mitosis occurring at 57 mm bud length, megasporocyte mitosis occurring at 69 mm bud length, the final mitosis which yields the eight nuclei of the mature embryo sac completed at anthesis or shortly thereafter, ribosomal RNA production occurring at 50-65 mm bud length, and flowers starting to open at 150 mm bud length (Erickson, 1948). Dry and fresh weight per style are lower just before anthesis, at the same time the protein is still relatively high.

Styles the day of anthesis probably have the highest metabolic activity of any age style. During this day, the

flower opens; the dry and fresh weight and total amount of protein increase greatly; the incompatibility reaction begins; proteins and RNA are being made for the self-incompatibility reaction; stigmatic, and stylar exudate form; and the callose lines the stylar canal.

During the 24 hr after anthesis, the styles would not have the high level of metabolic activity as in styles the day of anthesis, since there are decreases in the dry and fresh weight and also there is a sharp decrease in the total amount of protein. But at the same time, there probably would not be increased degradative metabolism.

The period from anthesis up to the fifth day after anthesis could be a period of steady state conditions in the lily style, since the total amount of protein remains at a relatively high value. During this time, the increase in protein from synthesis is accompanied probably by an equal amount of degradation. The type of protein made during this period probably plays an important role in the incompatibility reaction, and directly or indirectly in pollen tube feeding.

The second and sharpest decrease in the total amount of protein, after the fifth day after anthesis, is probably a result of a great increase of protein degradation because of the onset of senescence. The flower during this period becomes brown and the color becomes darker as the flower ages and the tepals collapse.

A protein increase as the incompatibility reaction begins, a protein decrease and leveling off as the incompatibility reaction remains strong, then a protein decrease as the incompatibility reaction disappears would appear to confirm the importance of protein both in the self-incompatibility reaction and in the general life of the style.

We wanted to see how our results correlated by using two methods, a Coomassie Brilliant Blue G-250 (CB) method that is based on the CB dye binding to protein, and the second method a modified microkjeldahl procedure that is based on a determination of the total nitrogen with the assumption of a direct ratio of nitrogen to protein.

The nitrogen value obtained is usually multiplied by 6.25 to give the crude protein content of the tissue. This conversion factor is derived from the observation that the average amino acid from which the proteins are synthesized contains approximately 16% nitrogen. This factor may not be accurate for all plant tissue. For example, 37-64% of the nitrogen in the potato tuber is non-protein, with as much as 32-46% in the form of amide nitrogen (Vigne and Li 1975). Also in addition to the twenty common amino acids encountered in protein hydrolysates, there are other amino acids present that have been collectively characterized as the non-protein amino acids.

We found the total amount of protein determined by the CB method was almost a mirror image of the total amount of protein as determined by microkjeldahl. The dramatic decrease in the total amount of protein as the the style ages is probably due to protein degradation in the styles with the accumulation of soluble amino acids. Protein degradation and amino acid accumulation could also occur in the tepals (sepals and petals) and the stem part that are still connected to the flower during senescence. During senescence the microkjeldahl assay cannot predict the total amount of protein and must remain only an approximation since a high percentage of nitrogenous components then are non protein, yet are converted to ammonia during nitrogen assay. The dramatic increase in total amount of nitrogen in the style may come from the transfer into the styles of the amino acids forming in the tepals (sepals and petals) and the stem part. Since the style remains active longer than the other flower parts, the accumulation continues until there is no more nitrogenous components that can be transfered to and accumulated in the styles.

During senescence, the amino acids that accumulate in detached leaves with time are increasingly utilized as respiratory substrates. While the carbon skeletons are used in respiratory metabolism, the nitrogenous components appear to accumulate in the amido residues of

glutamine and asparagine that is dependent on the presence of the available carbohydrate supply. Also it is consistent with an extensive proteolysis (Martin and Thimann, 1972).

In the heat treatment experiment the increase of the total amount of protein at 24 hr and 48 hr incubation may be due to an increase in synthesis in heat-treated styles due to heat treatment, with the slight degradation in non-heat-treated styles due to the increasing time of incubation of the detached styles. This protein synthesis at 24 hr and 48 hr in heat-treated styles may not have a relation to the special stylar substance or incompatibility reaction, for two reasons.

One of the effects of high-temperature treatments is the release of a special stylar substance into the stylar canal by the stylar canal cells of the lily. This secretion makes the incompatible pollen tubes grow as compatible for, when this material is flushed out of heat treated style, both types of pollen tubes grow as incompatible (Ascher, 1973). Once flushed out, special stylar substance does not accumulate in the stylar canal again giving compatible type growth.

If we self-pollinated the heat-treated styles after 48 hr incubation, we would still get compatible pollen tubes. This means that there is no reactivation of the stylar incompatibility occurring in pistils during the 48 hr following the heat-induced inactivation. Also the

heat-induced inactivation of stylar incompatibility is achieved as early as 39 hr prior to anthesis. Sensitivity of the stylar incompatibility to heat is independent of anthesis and stigma exudate formation (Hopper, Peloquin, 1967).

The next step in incompatibility protein research for the future is qualitative analysis of the protein to determine the natural of the incompatibility and the special stylar substance.

LITERATURE CITED

1. Ascher, P.D.: A gene action model to explain gametophytic self-incompatibility. Euphytica 15, 179-183 (1966)

2. Ascher, P.D.: Temperature and the self-incompatibility reaction in Lilium longiflorum Thunb. J. Amer. Soc. Hort. Sci. 95 (5), 586-588 (1970)

3. Ascher, P.D.: The influence of RNA-synthesis inhibitors on in vivo pollen tube growth and the self-incompatibility reaction in Lilium longiflorum Thunb. Theoret. Appl. Genet. 41, 75-78 (1971)

4. Ascher, P.D.: The effect of pre-pollination stylar flush on pollen tube growth in heat-treated styles of Lilium longiflorum Thunb. Incompatibility Newsletter 3, 4-6 (1973)

5. Ascher, P.D.: The self-incompatibility reaction in detached styles of Lilium longiflorum Thunb. injected before pollination with 6-methylpurine or puromycin. Incompatibility Newsletter 4, 57-60 (1974)

6. Ascher, P.D.: Special stylar property required for compatible pollen tube growth in Lilium longiflorum Thunb. Bot. Gaz. 136, 317-321 (1975)

7. Ascher, P.D.; Drewlow L.W.: The effect of cycloheximide and 6-methylpurine on in vivo compatible and incompatible pollen tube growth in Lilium longiflorum. Theoret. Appl. Genet. 40, 173-175 (1970a)

8. Ascher, P.D.; Drewlow L.W.: Stigmatic Exudate: A potential carrier of exogenous substances into the style of Lilium longiflorum Thunb. J. Amer. Soc. Sci. 95 (6), 706-708 (1970b)

9. Ascher, P.D.; Drewlow, L.W: The effect of dimethyl sulfoxide on compatible and incompatible pollen tube growth in Lilium longiflorum Thunb. styles. Theoret. Appl. Genet. 132, 146-148 (1971)

10. Ascher, P.D.; Peloquin, S.J.: Influence of temperature on incompatible and compatible pollen tube growth in Lilium longiflorum. Can. J. Genet. Cytol. 8, 661-664 (1966a)

11. Ascher, P.D.; Peloquin, S.J.: Effect of floral aging on the growth of compatible and incompatible pollen tubes in Lilium longiflorum. Amer. J. Bot. 53, 99-102 (1966b)

12. Beevers, Leonard,: Nitrogen Metabolism in plants. 25 Hill Street, London WiX 8LL: Edward Arnold 1976

13. Bradford, M: A rapid and sensitive method for quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. Analytical Biochemistry 72, 248-245 (1976)

14. Campbell, R.J.; Ascher, P.D.: Reduced pollen tube length in Lilium longiflorum Thunb. styles injected at pollination with phenol-extracted nucleic acids. Incompatibility Newsletter 4, 33-37 (1974)

15. Campbell, R.J.; Ascher, P.D.: Incorporation of radioactive label into nucleic acids of compatible and incompatible pollen tubes of Lilium longiflorum Thunb. Theoret. Appl. Genet. 46, 143-148 (1975a)

16. Campbell, R.J.; Ascher, P.D.: Radioactive stigmatic exudate production by Lilium longiflorum Thunb. styles labeled with 5-^{3}H-Uridine. Incompatibility Newsletter 5, 76-81 (1975b)

17. Campbell, R.J.; Ascher, P.D.: Incorporation of label from radioactive uridine into the stylar nucleic acids of Lilium longiflorum Thunb. as affected by heat, 6-methyputine and actinomycin D. Theoret. Appl. Genet. 47, 215-226 (1976)

18. Dashek, W.V.; Thomas, H.R.; Rosen, W.G.: Secretory cells of lily pistils. II. E.M. cytochemistry of canal cells. Amer. J. Bot. 58, 909-920 (1971)

19. Donk, J.A.W. Van der: Differential synthesis of RNA in self- and cross-pollinated styles of Petunia hybrida L. Molec. Gen. Genet. 131, 1-8 (1974a)

20. Donk, J.A.W. Van der: Synthesis of RNA and protein as a function of time and type of pollen tube-style interaction in Petunia hybrida L. Molec. Gen. Genet. 134, 93-98 (1974b)

21. Frankel, R.; E. Galun: Pollination Mechanisms, reproduction and plant Breeding. Berlin, Heidelberg, New York: Springer 1977

22. Hopper, J.E.; Ascher, P.D.; Peloquin, S.J.: Inactivation of self-incompatibility following temperature pretreatment of styles in Lilium longiflorum. Euphytica 16, 215-220 (1967)

23. Hopper, J.E.; Ascher, P.D.; Peloquin, S.J.: Analysis of stylar self-incompatibility competence by use of heat induced inactivation. Theoret. Appl. Genet. 47, 291-220 (1976)

24. Kuraishi, S.: The effect of kinetin on protein level of Brassica leaf disks. Physiologia Pl. 21, 78-83 (1968)

25. Linder, R.; Linskens, H.F.: Evaluation des acids aminés dans le style d' Oenothera missouriensis vieige, autopollinisé et xénopollinise. Theoret. Appl. Genet. 42, 125-129 (1972)

26. Linskens, H.F.: Biochemistry of incompatibility Proc. 11th Intern. Congr. Gent. The Hague, 1963. In: Genetics Today, Geets, S.J. (ed), 3, 621-676 (1965)

27. Martin, D.; Thimann, K.V.: The role of protein synthesis in the senescence of leaves. I. The formation of protease. Pl. Physiol, Lancaster. 49, 64-71 (1972)

28. Nasrallah, M.E.; Barber, J.T.; Wallace, D.H.: Self-incompatibility proteins in plants: Detection, genetics and possible mode of action. Heredity 24, 23-27 (1969)

29. Nettancout, D. de: Incompatibility in Angiosperms. Berlin, Heidelberg, New York: Springer 1977.

30. Rosen, W.G.; Thomas, H.R.: Secretory cells of lily pistils. I. Fine structure and function. Amer. J. Bot. 57, 1108-1114 (1970)

31. Snyder, J.C.; Desborough, S.L.: Rapid estimation of potato tuber total protein content with Coomassie Brilliant blue G-250. Theoret. Appl. Genet. 52, 1-6 (1978)

32. Tupy, J.: Investigation of free amino acids in cross, self- and non pollinated pistils of Nicotiana alata. Biologia Plantarum (Praha) 3 (1), 47-64 (1961)

33. Vigue, J.; Li, P.H.: Correlation between methods to determine the protein content of potato tuber. HortScience 10, 624-627 (1975)

34. Yemm, E.W.: Respiration of barley plants. IV. Protein catabolism and the formation of amides in starving leaves. Proc. R. Soc. London Sci. B, 136 632-649 (1950)

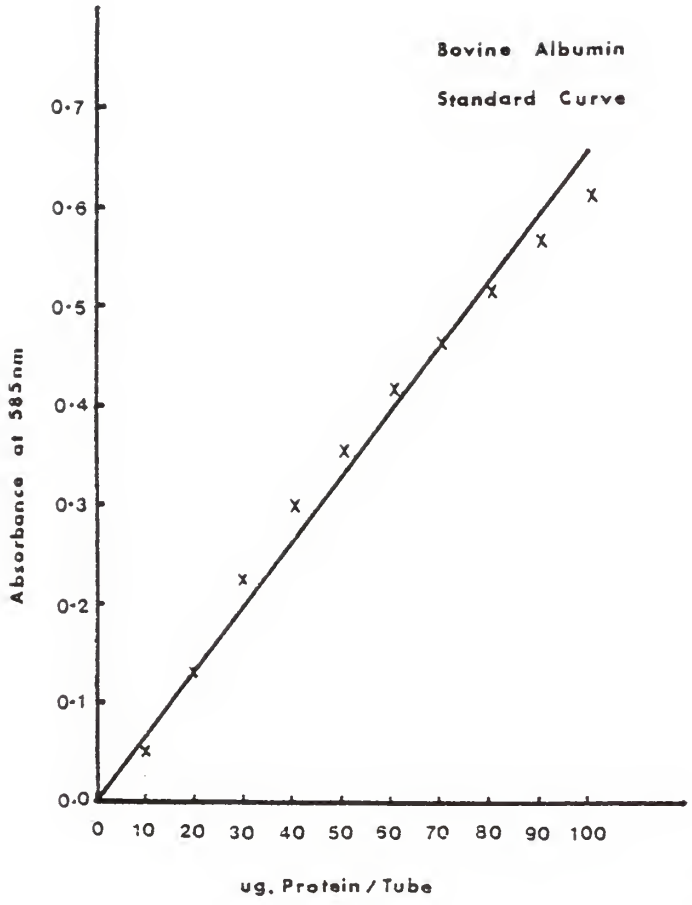

Bovine Albumin
Standard Curve
Absorbance at 585nm
0·0
0·1
0·2
0·3
0·4
0·5
0·6
0·7
0 10 20 30 40 50 60 70 80 90 100
ug. Protein / Tube

Fig. (1)

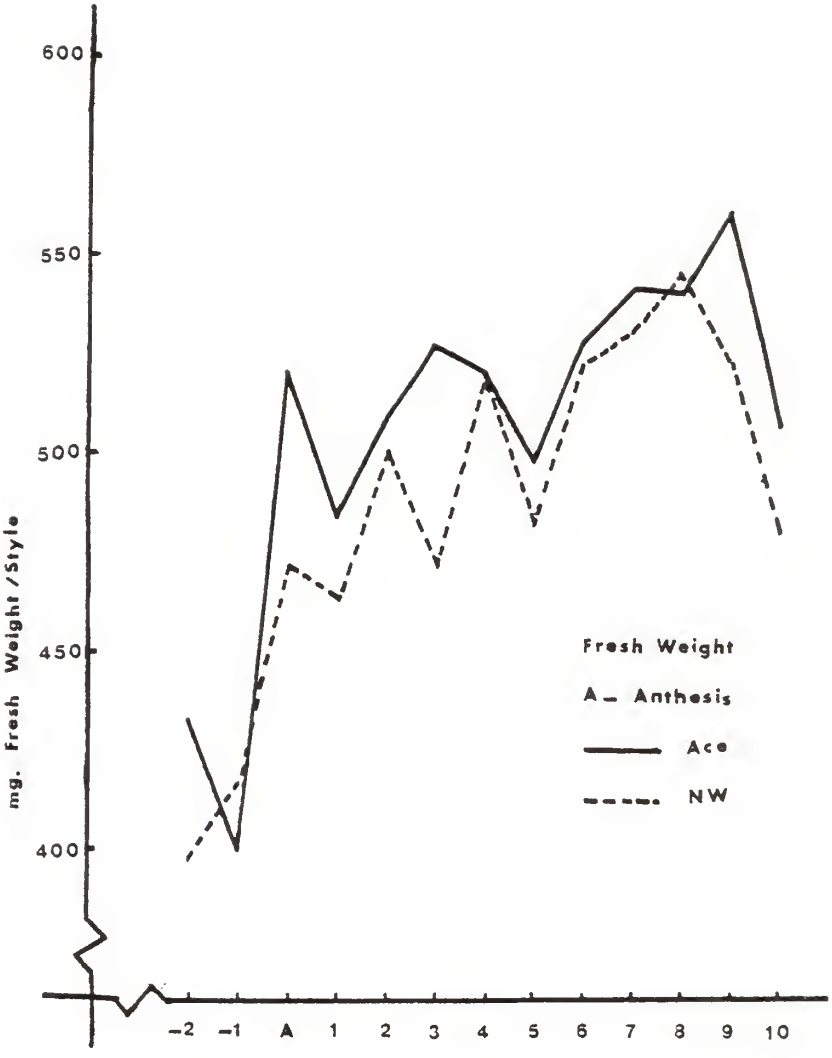

600
550
500
450
400
mg. Fresh Weight /Style
Fresh Weight
A – Anthesis
Ace
NW
–2 –1 A 1 2 3 4 5 6 7 8 9 10
Floral Age in Days

Fig.(2)

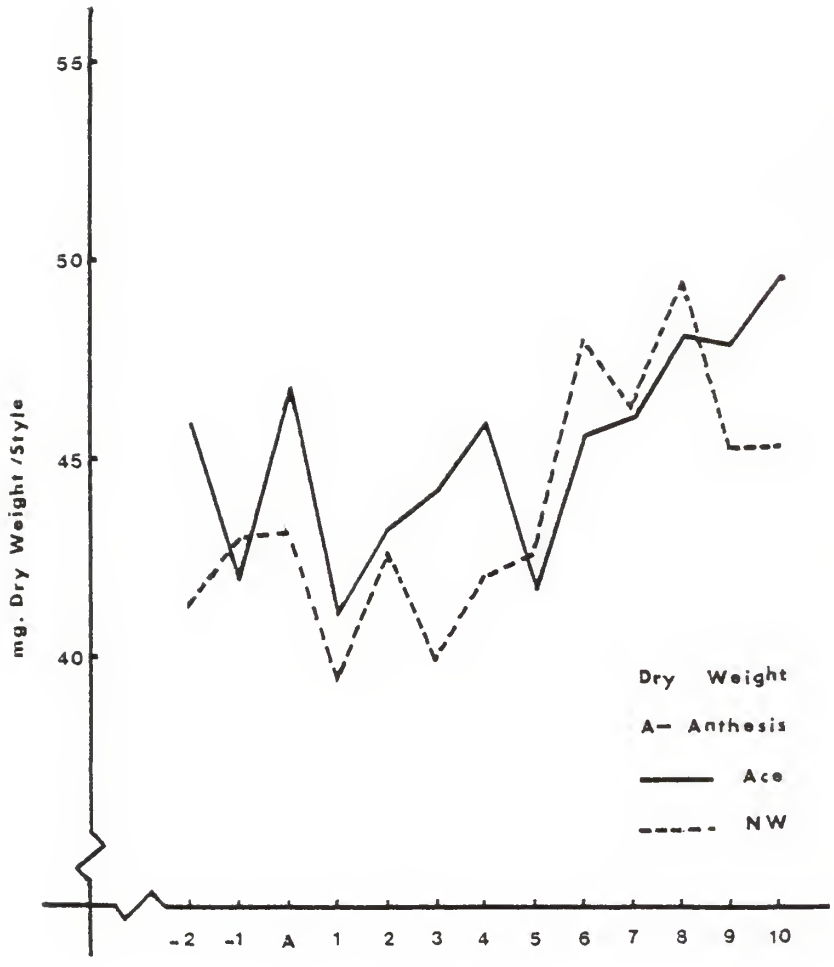

55
50
45
40
mg. Dry Weight /Style
-2
-1
A
1
2
3
4
5
6
7
8
9
10
Floral Age in Days
Dry Weight
A— Anthesis
Ace
NW

Fig.(3)

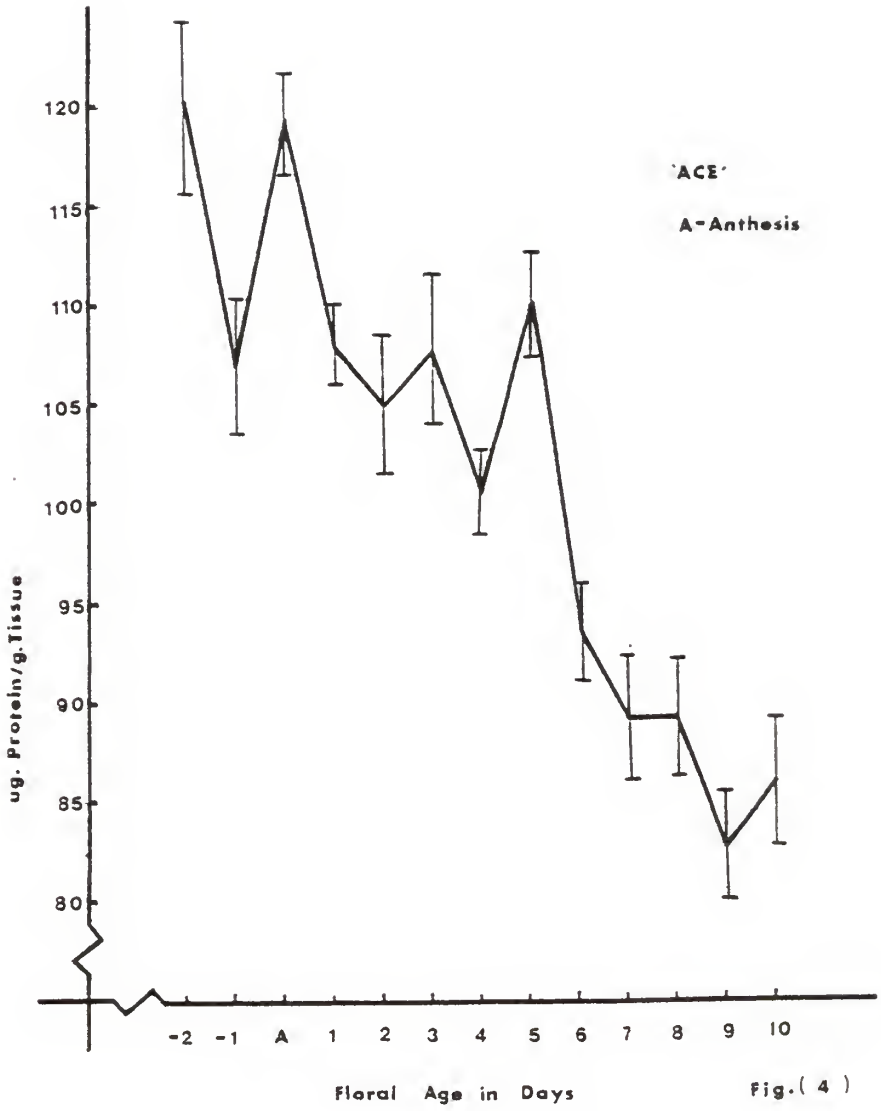

'ACE'
A-Anthesis
ug. Protein/g.Tissue
120
115
110
105
100
95
90
85
80
-2
-1
A
1
2
3
4
5
6
7
8
9
10
Floral Age in Days

Fig.(4)

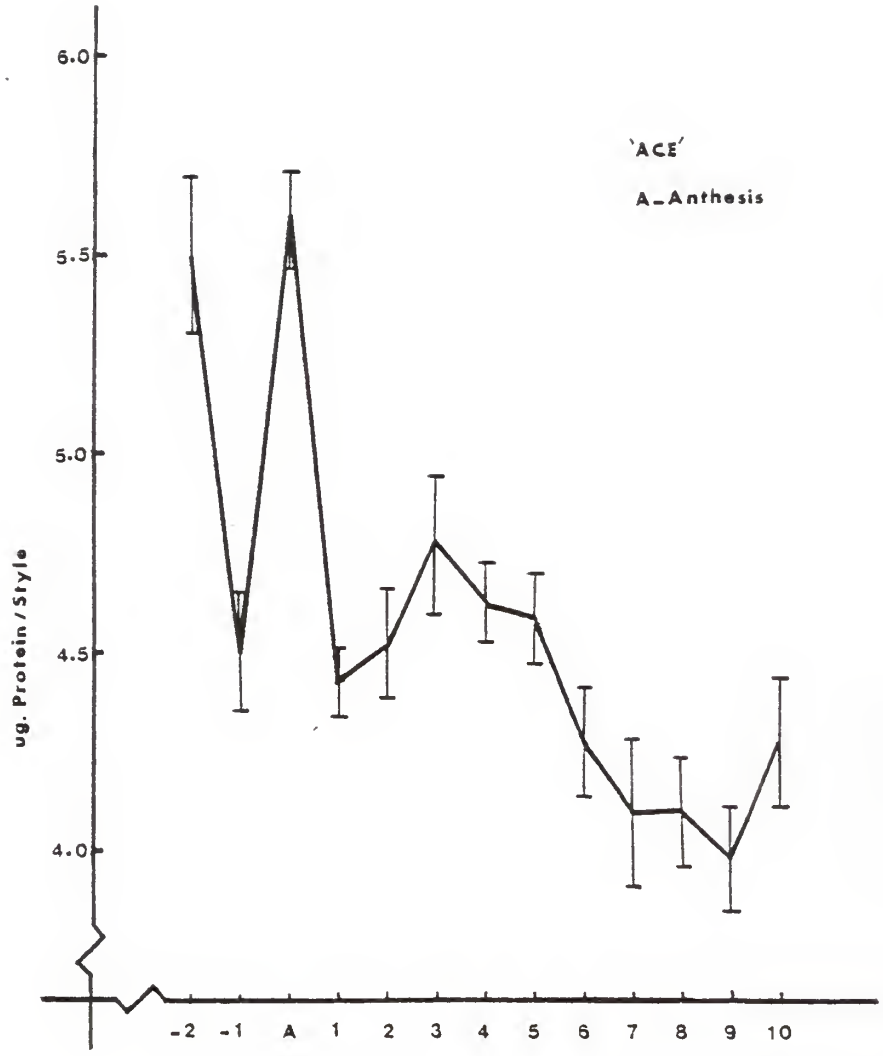

'ACE'
A-Anthesis
6.0
5.5
5.0
4.5
4.0
ug. Protein / Style
-2 -1 A 1 2 3 4 5 6 7 8 9 10
Floral Age in Days

Fig. (5)

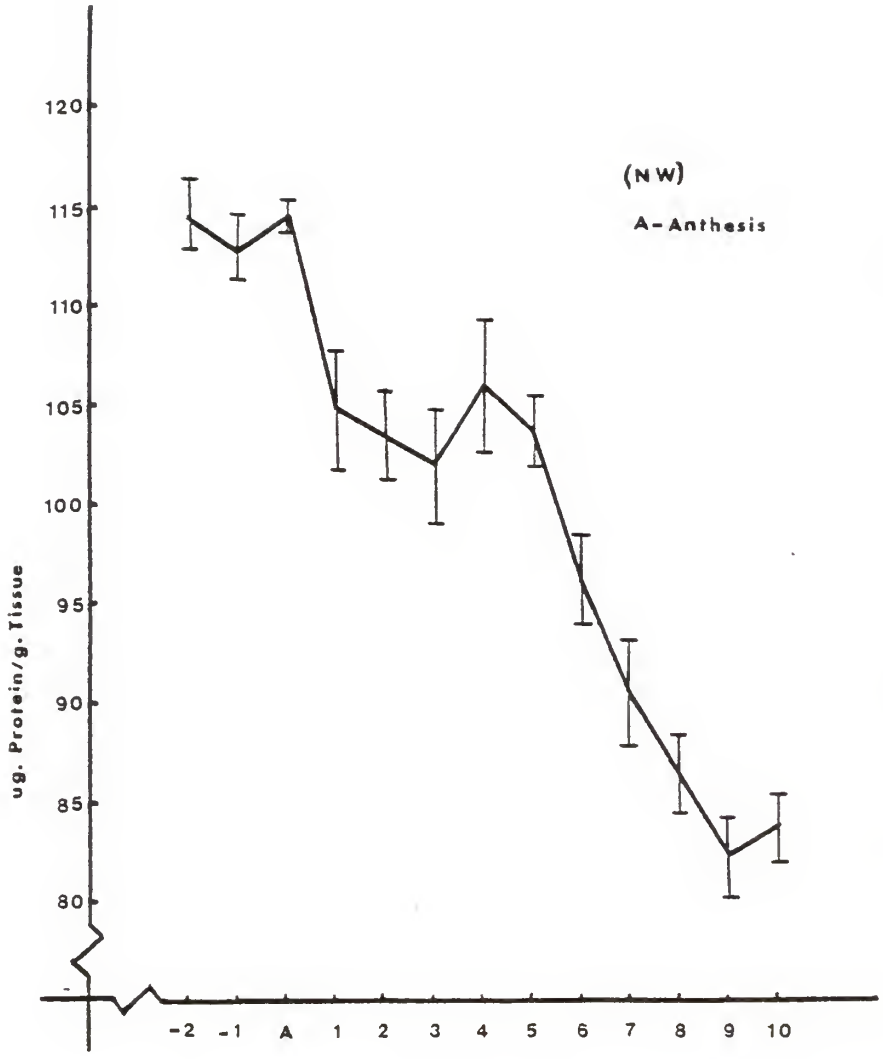

(N W)
A- Anthesis
ug. Protein/g. Tissue
120
115
110
105
100
95
90
85
80
-2
-1
A
1
2
3
4
5
6
7
8
9
10
Floral Age in Days

Fig. (6)

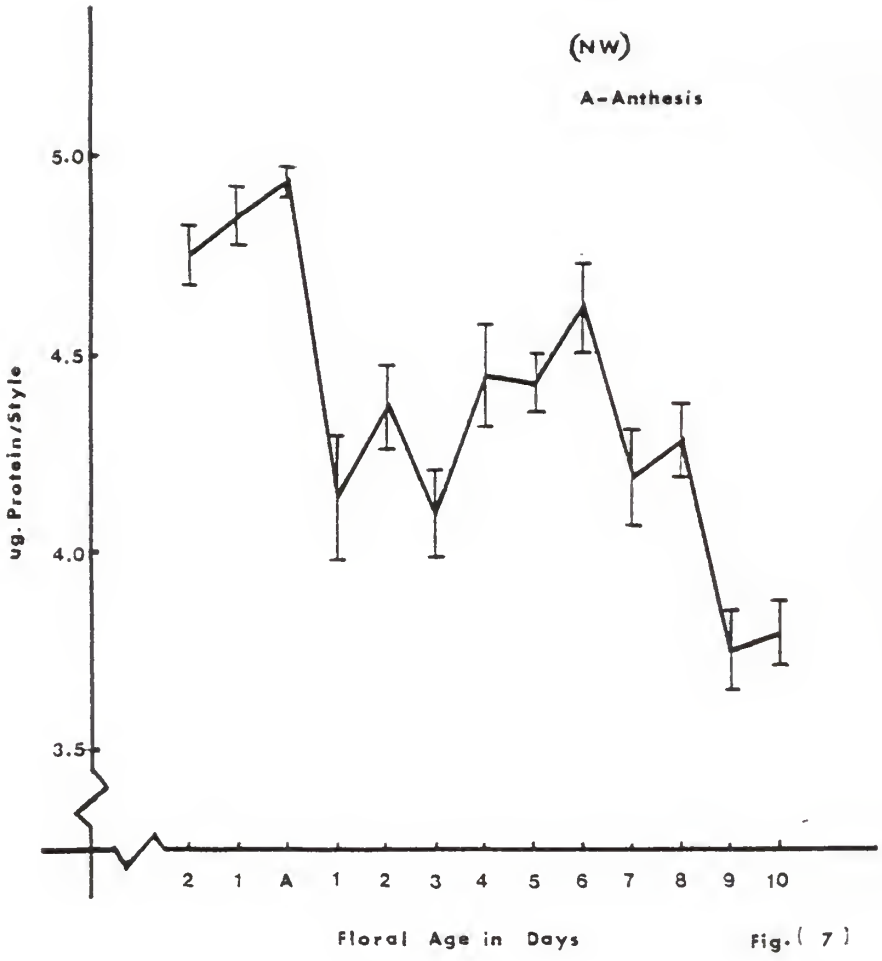

(NW)
A-Anthesis
5.0
4.5
4.0
3.5
ug. Protein/Style
2 1 A 1 2 3 4 5 6 7 8 9 10
Floral Age in Days

Fig.(7)

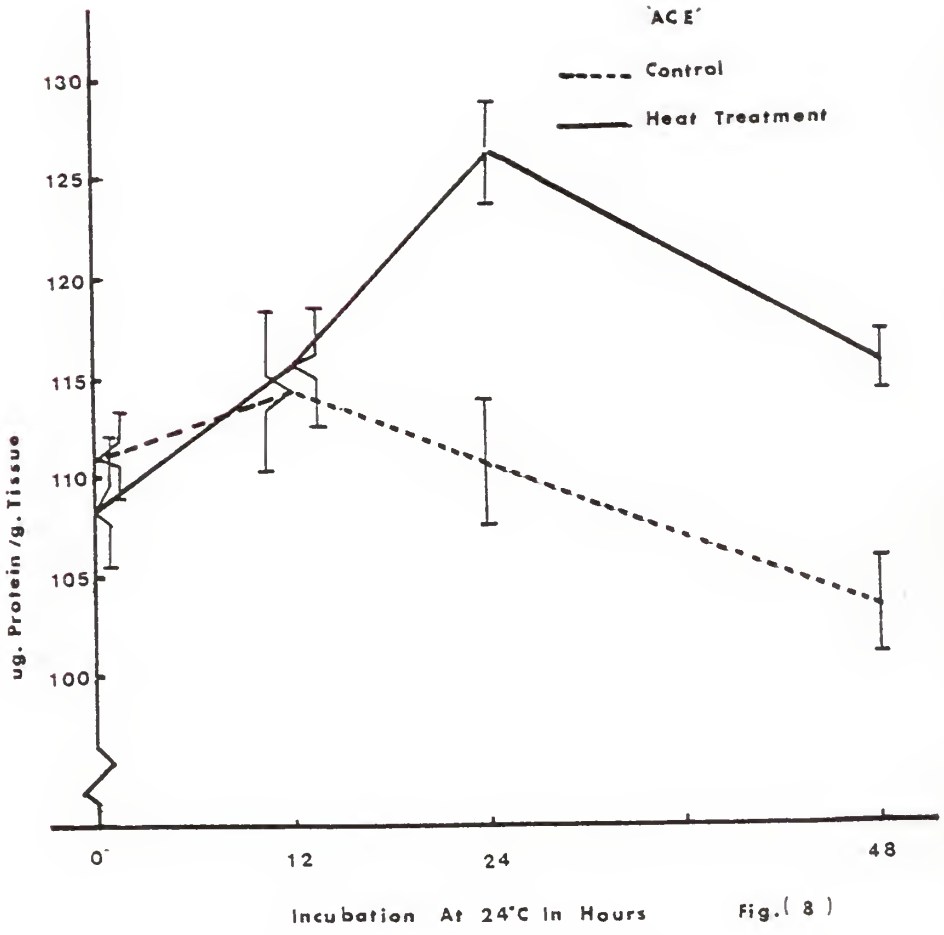

'ACE'
Control
Heat Treatment
130
125
120
115
110
105
100
ug. Protein /g. Tissue
0
12
24
48
Incubation At 24°C In Hours

Fig.(8)

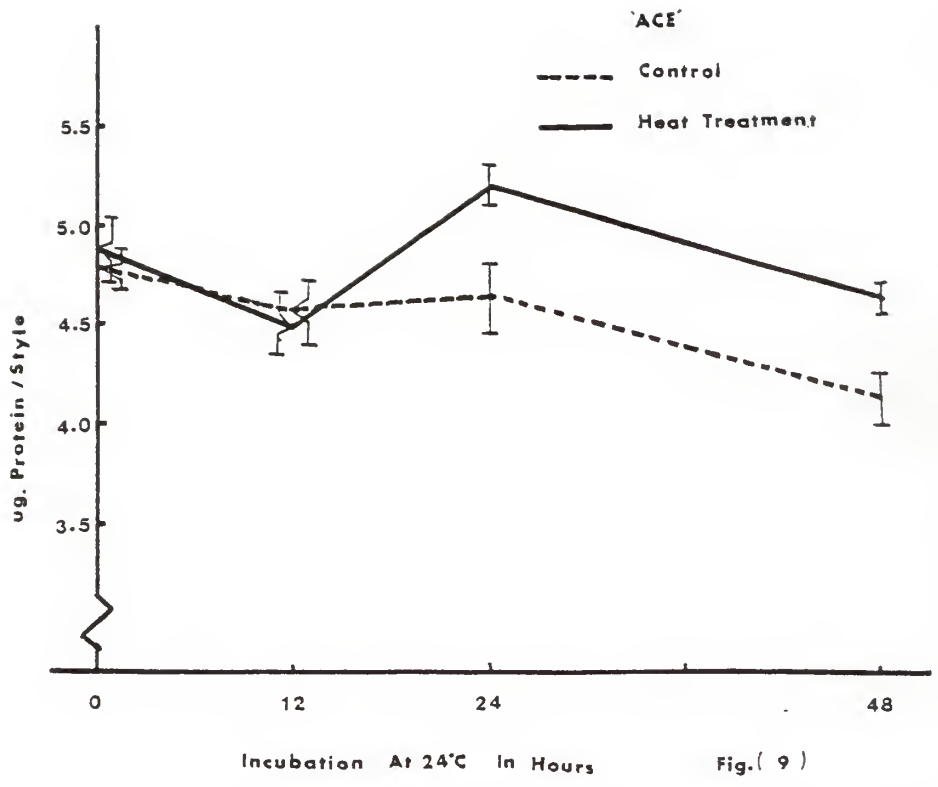

'ACE'
Control
Heat Treatment
5.5
5.0
4.5
4.0
3.5
ug. Protein / Style
0
12
24
48
Incubation At 24°C In Hours

Fig.(9)

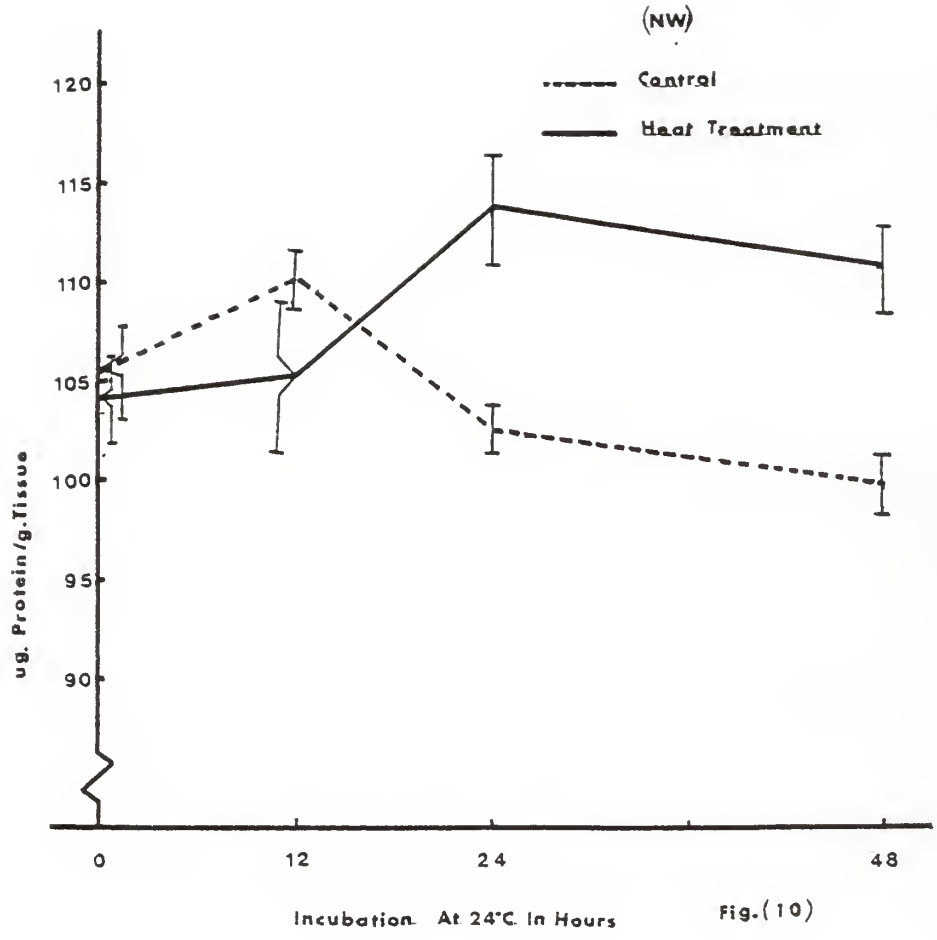

(NW)
Control
Heat Treatment
120
115
110
105
100
95
90
ug. Protein/g.Tissue
0
12
24
48
Incubation At 24°C In Hours

Fig.(10)

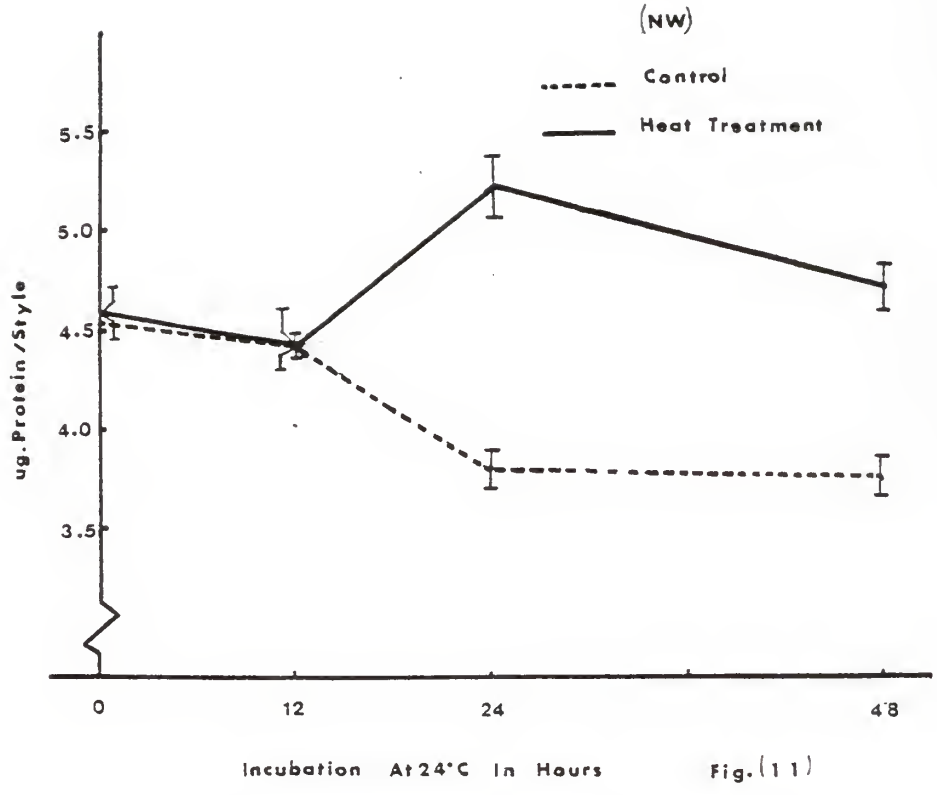

(NW)
Control
Heat Treatment
5.5
5.0
4.5
4.0
3.5
ug. Protein / Style
0
12
24
48
Incubation At 24°C In Hours

Fig. (11)

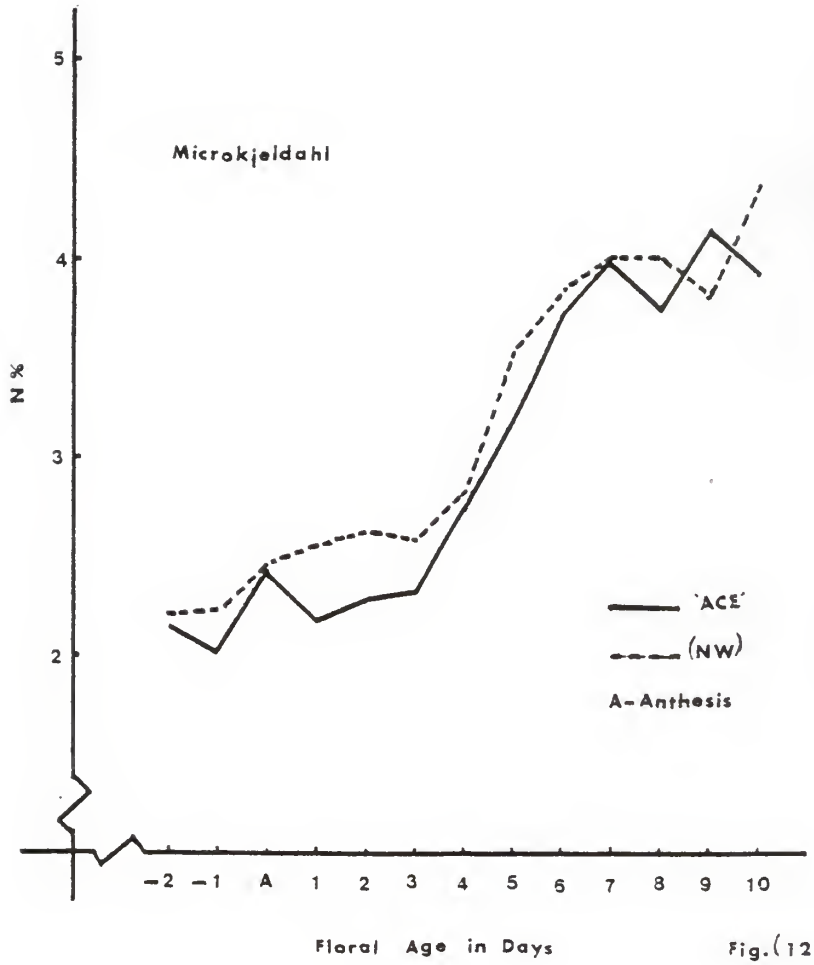

Microkjeldahl
N %
5
4
3
2
'ACE'
(NW)
A-Anthesis
-2 -1 A 1 2 3 4 5 6 7 8 9 10
Floral Age in Days

Fig.(12)

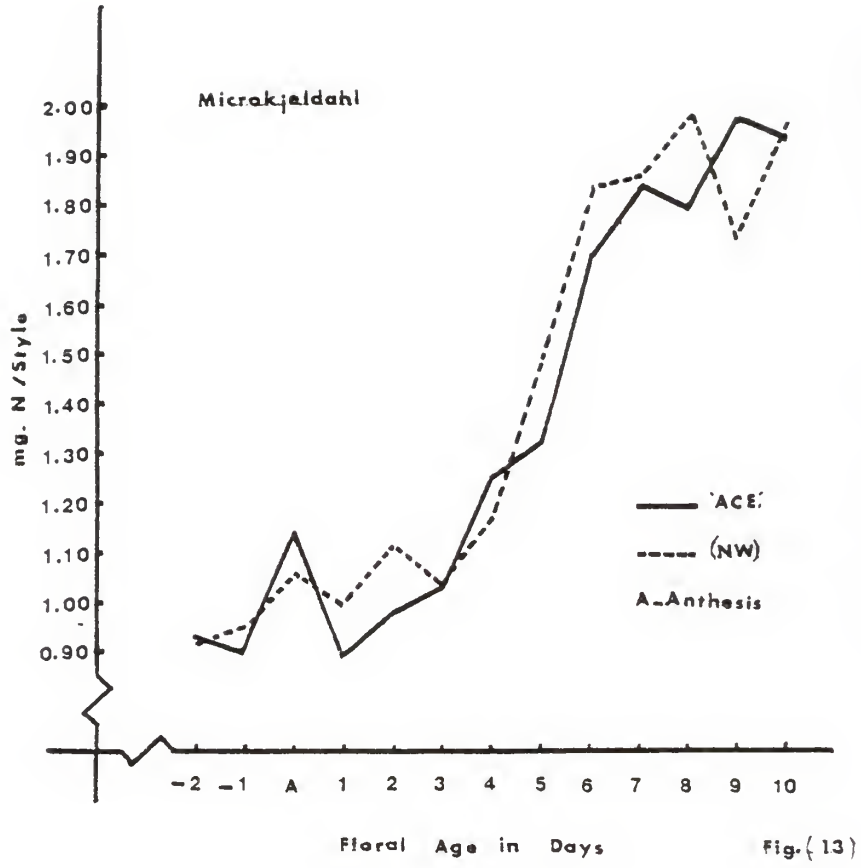

Micrakjeldahl
2.00
1.90
1.80
1.70
1.60
1.50
1.40
1.30
1.20
1.10
1.00
0.90
mg. N /Style
'ACE'
(NW)
A-Anthesis
-2 -1 A 1 2 3 4 5 6 7 8 9 10
Floral Age in Days

Fig.(13)

TOTAL AMOUNT OF PROTEIN IN *LILIUM LONGIFLORUM* THUNB., EASTER LILY, STYLES FROM 12 DAYS PRE-ANTHESIS TO 10 DAYS POST-ANTHESIS

by

ABDULLAH SALIH AL-GHAMDI

B. S., University of Riyad, Riyad, 1975

AN ABSTRACT OF A MASTER'S THESIS

submitted in partial fulfillment of the

requirements for the degree

MASTER OF SCIENCE

Department of Horticulture

KANSAS STATE UNIVERSITY
Manhattan, Kansas
1979

Proteins were extracted using 2N NaOH from freeze dried powder obtained from Lilium longiflorum Thunb., Easter lily, styles from 2 days pre-anthesis until 10 days post-anthesis, and analyzed by using a colorimetric test; the powder was also analyzed for nitrogen by microkjeldahl.

Fresh and dry stylar weight increased in different ways as the flowers aged. Fresh weight increased most rapidly during the day preceding anthesis; increased less sharply after that, peaking at 9-10 days after anthesis; and then rapidly declined. Dry weight, about 9% of style fresh weight, was more variable than fresh weight, with a slight increase in dry weight the day of anthesis and after the fifth day after anthesis.

Total protein decreased. The largest amount of protein was in the two days pre-anthesis to day of anthesis styles. A sharp decrease in total amount of protein occurred from the day of anthesis to one day later. From one day after anthesis until the fifth day, the total protein seemed to plateau. From the fifth day until the ninth day the total protein decreased sharply.

Total protein in non heat-treated styles began at the same protein level expected for 1 day old styles, then showed the plateauing of protein across time as in 2 and 3 day old styles. From 24 hr up to 48 hr incubation there was a decrease in the amount of stylar protein.

The amount of protein in heat-treated styles was the same at 0 time and 12 hr as for non heat-treated styles but increased until it reached a high value at 24 hr incubation. By 48 hr, the amount decreased.

In the microkjeldahl assay for determining total nitrogen in styles, nitrogen amounts started at a relatively low level and increased slightly from two days pre-anthesis to 3 days post-anthesis. From the third day until the seventh day after anthesis, there was a sharp increase. % nitrogen then plateaued with increased variability.